B8 Cont

concentration of 10 μM substrate. A 0 time fluorescence reading (320 excitation; 390 emission) is immediately taken and subsequent readings are taken every fifteen minutes at room temperature with a PerSeptive Biosystems CytoFluor Multi-Well Plate Reader with the gain at 90 units.

In the Claim:

Please replace Claims 60-61 with the following claims:

B9

60. (Amended) A method of inhibiting the cleavage of TNF-α from cell membranes without inhibiting MMP-1 in a mammal, comprising administering to such mammal an effective amount of a small molecule that possesses at least 100 fold IC_{50} selectivity for TACE over MMP-1; wherein MMP-1 activity is determined by an MMP-1 in vitro assay and wherein TACE activity is determined by a human monocyte assay.

61. (Amended) A method of inhibiting the cleavage of TNF-α from cell membranes without inhibiting MMP-1 in a mammal comprising administering to such mammal an effective amount of a hydroxamic acid compound that possesses at least 100 fold IC_{50} selectivity for TACE over MMP-1; wherein MMP-1 activity is determined by an MMP-1 in vitro assay and wherein TACE activity is determined by a human monocyte assay.

Please add the following new Claims 80-81:

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80. (New) A method of inhibiting the cleavage of TNF-α from cell membranes without inhibiting MMP-1 in a mammal, comprising administering to such mammal an effective amount of a small molecule that possesses at least 500 fold IC_{50} selectivity for TACE over MMP-1; wherein MMP-1 activity is determined by an MMP-1 in vitro assay and wherein TACE activity is determined by a human monocyte assay.

81. (New) A method of inhibiting the cleavage of TNF-α from cell membranes without inhibiting MMP-1 in a mammal comprising administering to such mammal an effective amount of a hydroxamic acid compound that possesses at least 500 fold IC_{50} selectivity for TACE over MMP-1; wherein MMP-1 activity is determined by an MMP-1 in vitro assay and wherein TACE activity is determined by a human monocyte assay.

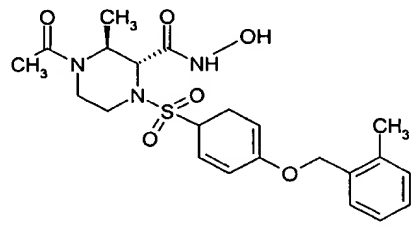
VERSION WITH MARKINGS TO SHOW CHANGES MADE - DO NOT ENTER

In the Specification:

Please delete the paragraph at page 41, lines 26-32, and replace such deleted paragraph with the following paragraph:

A model peptidic TNF-α substrate (LY-LeucineAlanineGlutamineAlanineValine-ArginineSerine-SerineLysine(CMTR)-Arginine (LY=Lucifer Yellow; CMTR= 5-carboxytetramethyl Rhodamine)) (hereinafter SEQ ID No: 4) was prepared and the concentration estimated by absorbance at 560 nm (E_{560}, 60,000 M-1CM-1) according to the method of Geoghegan, KF, "Improved method for converting an unmodified peptide to an energy-transfer substrate for a proteinase." Bioconjugate Chem. 7, 385-391 (1995). This peptide encompasses the cleavage cite on pro-TNF which is cleaved in vivo by TACE.

Please delete the paragraph at page 43, Table A, Row 3, and replace such deleted row with the following row:

Chiral 	6.2	1600	258	3

Please delete the paragraph at page 45, lines 15-18, and replace such deleted paragraph with the following paragraph:

Substrate (DNP-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(NMA)-NH_2) (hereinafter SEQ ID No: 2) is made as a 5 mM stock in dimethyl sulfoxide and then diluted to 20 mM in assay buffer. The assay is initiated by the addition of 50 µl substrate per well of the microfluor plate to give a final concentration of 10 µM.

Please delete the paragraph at page 45, lines 33-35, and replace such deleted paragraph with the following paragraph:

Inhibition of gelatinase activity is assayed using the [(DNP-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(NMA)-NH_2)] SEQ ID No: 2 substrate (10 µM) under the same conditions as inhibition of human collagenase (MMP-1).

Please delete the paragraph at page 46, lines 11-15, and replace such deleted paragraph with the following paragraph:

Inhibition of stromelysin activity is based on a modified spectrophotometric assay described by Weingarten and Feder (Weingarten, H. and Feder, J., Spectrophotometric Assay for Vertebrate Collagenase, Anal. Biochem. 147, 437-440 (1985)). Hydrolysis of the thio peptolide substrate [Ac-Pro-Leu-Gly-SCH[$CH_2CH(CH_3)_2$]CO-Leu-Gly-OC_2H_5] (hereinafter SEQ ID No: 3) yields a mercaptan fragment that can be monitored in the presence of Ellman's reagent.

Please delete the paragraph at page 47, lines 12-15, and replace such deleted paragraph with the following paragraph:

Substrate (Dnp-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(NMA)-NH_2) (hereinafter SEQ ID No: 2) is prepared as for inhibition of human collagenase (MMP-1) and 50 ml is added to each well to give a final assay concentration of 10 µM. Fluorescence readings (360 nM excitation; 450 emission) are taken at time 0 and every 5 minutes for 1 hour.

Please delete the paragraph at page 50, lines 2-4, and replace such deleted paragraph with the following paragraph:

Inhibition of 92 kD gelatinase (MMP-9) activity is assayed using the Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 (hereinafter SEQ ID No: 1) substrate (10 µM) under similar conditions as described above for the inhibition of human collagenase (MMP-1).

Please delete the paragraph at page 50, lines 22-27, and replace such deleted paragraph with the following paragraph:

A five mM dimethylsulfoxide stock solution of substrate SEQ ID No: 1 [(Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2)] is diluted in assay buffer to 20 μM. The assay is initiated by addition of 50 μL of diluted substrate yielding a final assay concentration of 10 μM substrate. A 0 time fluorescence reading (320 excitation; 390 emission) is immediately taken and subsequent readings are taken every fifteen minutes at room temperature with a PerSeptive Biosystems CytoFluor Multi-Well Plate Reader with the gain at 90 units

In the Claim:

Please replace Claims 60-61 with the following claims:

60. (Amended) A method of inhibiting the cleavage of TNF-α from cell membranes without inhibiting MMP-1 in a mammal, comprising administering to such mammal an effective amount of a small molecule that [inhibits the TNF-α proteolytic activity of TACE without inhibiting MMP-1] possesses at least 100 fold IC_{50} selectivity for TACE over MMP-1; wherein MMP-1 activity is determined by an MMP-1 in vitro assay and wherein TACE activity is determined by a human monocyte assay.

61. (Amended) A method of inhibiting the cleavage of TNF-α from cell membranes without inhibiting MMP-1 in a mammal comprising administering to such mammal an effective amount of a hydroxamic acid compound that [inhibits the TNF-α proteolytic activity of TACE without inhibiting MMP-1] possesses at least 100 fold IC_{50} selectivity for TACE over MMP-1; wherein MMP-1 activity is determined by an MMP-1 in vitro assay and wherein TACE activity is determined by a human monocyte assay.

Please add the following new Claims 80-81:

80. (New) A method of inhibiting the cleavage of TNF-α from cell membranes without inhibiting MMP-1 in a mammal, comprising administering to such mammal an effective amount of a small molecule that possesses at least 500 fold IC_{50} selectivity for TACE over MMP-1; wherein MMP-1 activity is determined by an MMP-1 in vitro assay and wherein TACE activity is determined by a human monocyte assay.

81. (New) A method of inhibiting the cleavage of TNF-α from cell membranes without inhibiting MMP-1 in a mammal comprising administering to such mammal an effective amount of a hydroxamic acid compound that possesses at least 500 fold IC_{50} selectivity for TACE over MMP-1; wherein MMP-1 activity is determined by an MMP-1 in vitro assay and wherein TACE activity is determined by a human monocyte assay.

REMARKS

Reconsideration of the above application is respectfully requested.

Claims 1-79 are pending in the application. Claims 1-59 and 62-79 are withdrawn from consideration as directed to non-elected subject matter. Claims 60-61 have been amended. New claims 80-81 have been added. These amendments add no new matter. Upon entry of this amendment, claims 60-61 and 80-81are pending. Entry of this amendment is respectfully requested.

I. FORMALITY OBJECTION TO THE SPECIFICATION

The Examiner has required that Applicants comply with the requirements of the sequence rules (37 C.F.R. 1.821 – 1.825). Specifically, the Examiner has requested that the numeric identifier <220> and <223> be further described when the numeric identifier <213> reads "artificial sequence".